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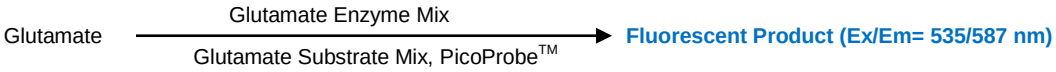
PicoProbe™ Glutamate Assay Kit (Fluorometric)

10/18

(Catalog # K413-100; 100 assays; Store at -20°C)

I. Introduction:

Glutamate (Glutamic Acid, Glu) is one of the most abundant proteogenic amino acids in humans. It is a key molecule in cellular metabolism. For example, Glutamate plays an important role both in the metabolism of some amino acids and disposal of excess nitrogen in the form of urea. In addition, Glutamate is the main excitatory neurotransmitter in mammalian nervous system and involved in learning, cognitive and memory processes. Glutamate signaling activates receptors which have been implicated in mental disorders (Schizophrenia), neurodevelopment disorders (autism) and neurodegenerative diseases like Alzheimer's and Parkinson's disease. BioVision's PicoProbe™ Glutamate Assay Kit (Fluorometric) allows for quantification of Glutamate in Biological Fluids and Tissues. The assay is based on an enzymatic reaction in which a fluorogenic probe is reduced producing a stable signal. The reduced fluorophore produces a strong signal (Ex/Em= 535/587 nm), which is directly proportional to the amount of Glutamate in samples. The assay is simple, reproducible, and can specifically detect as low as 5 pmol of Glutamate in a 100 µl reaction.



II. Applications:

- Measurement of Glutamate in various biological samples/preparations

III. Sample Type:

- Tissue Homogenates: Muscle, Liver, etc.
- Cell Lysates: Hela Cell Lysates, etc.
- Biological fluids: Serum, Cerebrospinal fluid (CSF), etc.

IV. Kit Contents:

Components	K413-100	Cap Code	Part Number
Glutamate Assay Buffer	50 ml	NM	K413-100-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K413-100-2
Glutamate Enzyme Mix	1 vial	Green	K413-100-3
Glutamate Substrate Mix	1 vial	Red	K413-100-4
Glutamate Standard (0.1 M)	0.1 ml	Yellow	K413-100-5

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (ELISA reader)
- 96-well white plate with flat bottom
- Dounce Tissue Homogenizer (Cat. #1998)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Upon opening, use within two months.

- **Glutamate Assay Buffer:** Store at either 4 ºC or -20 ºC. Bring to 37 ºC before use.
- **PicoProbe™:** Ready to use as supplied. Warm to room temperature before use. Store at -20 °C.
- **Glutamate Enzyme Mix & Glutamate Substrate Mix:** Reconstitute each vial with 220 µl of Glutamate Assay Buffer and mix thoroughly. Store at -20 °C. Avoid freeze/thaw. Use within two months. Keep on ice while in use.
- **Glutamate Standard:** Ready to use as supplied. Store at -20 ºC.

VII. Glutamate Assay Protocol:

1. **Sample Preparation: For Tissue and cells:** Homogenize tissue (10~20 mg) or pelleted cells (~1 x 10^6) with 400 µl ice-cold Glutamate Assay Buffer and keep on ice for 10 min. Centrifuge samples at 12,000 x *g* at 4 ºC for 10 min. and collect the supernatant. *If high concentration of glutamate are expected, dilute the supernatant 10-50 fold in Glutamate Assay Buffer*. Add 2-10 µl of Diluted Sample(s) into well(s) of a 96-well white plate. **For Serum and Cerebrospinal fluid (CSF):** Clarify samples by centrifugation at 10,000 x *g* at 4 ºC for 5 min in order to reduce turbidity and separate insoluble material. Prepare a 10-fold dilution of Serum in dH_2O (such as add 10 µl of Serum with 90 µl of dH_2O), CSF can be added directly. Add 2-10 µl of sample (CSF or Diluted Serum) into well(s) of a 96-well white plate. Adjust the volume of Sample(s) to **50 µl/well** with Glutamate Assay Buffer**. Sample Background Control:** Add 50 µl of Glutamate Assay Buffer in designated well(s) as Sample Background Control.

   Note:

   We suggest using 3-5 different amounts of unknown samples to ensure the readings are within the standard curve range and the changes of velocity are within the linear range.

2. **Standard Curve Preparation:** Prepare a 1 mM Glutamate Standard by adding 5 µl of 0.1 M Glutamate Standard to 495 µl Glutamate Assay Buffer, mix well; further prepare a 10 µM of Glutamate Standard by adding 5 µl of 1 mM Glutamate Standard to 495 µl Glutamate Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl of 10 µM (10 pmol/µl) Glutamate standard into a series of wells to generate 0, 20, 40, 60, 80, 100 pmol of Glutamate/well respectively. Adjust the volume to **50 µl/well** with Glutamate Assay Buffer.

3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed**.** For each well, prepare a total 50 µl Mix containing:

	Reaction Mix
Glutamate Assay Buffer	45 µl
Glutamate Enzyme Mix	2 µl
Glutamate Substrate Mix	2 µl
PicoProbe™	1 µl

Mix and add 50 µl of the Reaction Mix to each well containing the Glutamate Standard(s), Sample(s) and Sample Background Control, mix well. ***The total final reaction volume for each well will be 100 µl***.

4. **Measurement:** Measure fluorescence intensity (Ex/Em= 535/587 nm) in kinetic mode at 37°C for 60 min using a fluorescence microtiter plate reader. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding RFU for all Samples (R_{S1} and R_{S2}) and Sample Background Controls (R_{B1} and R_{B2}).

5. **Calculation:** Subtract 0 Standard reading from all Standard(s) readings. **Note:** It is normal to observe drifting in Glutamate Standards. **Extrapolate the linear portion of every time curve for each Standard, Background Control and Sample to the Y-axis to obtain the Y-intercept** (y-value at x= 0). Plot the Standard Curve using the corrected intercept values. Calculate the Glutamate of the test sample ΔY-Intercept= Y-Intercept$_{(RS2-RS1)}$ – Y-Intercept$_{(RB2-RB1)}$. Apply the ΔY-Intercept to the Standard Curve to get B pmol of Glutamate formed during the reaction time ($\Delta t = t_2 - t_1$).

Sample Glutamate Amount = B/ (V*P) x D = pmol/mg

Where: **B** is Glutamate amount from Standard Curve
V is Sample volume added into the reaction well (ml)
P is Initial Sample Concentration in mg-protein/ml (mgP/ml)
D is Sample Dilution Factor

Sample Glutamate Amount can also be expressed as µM (pmol/µl).

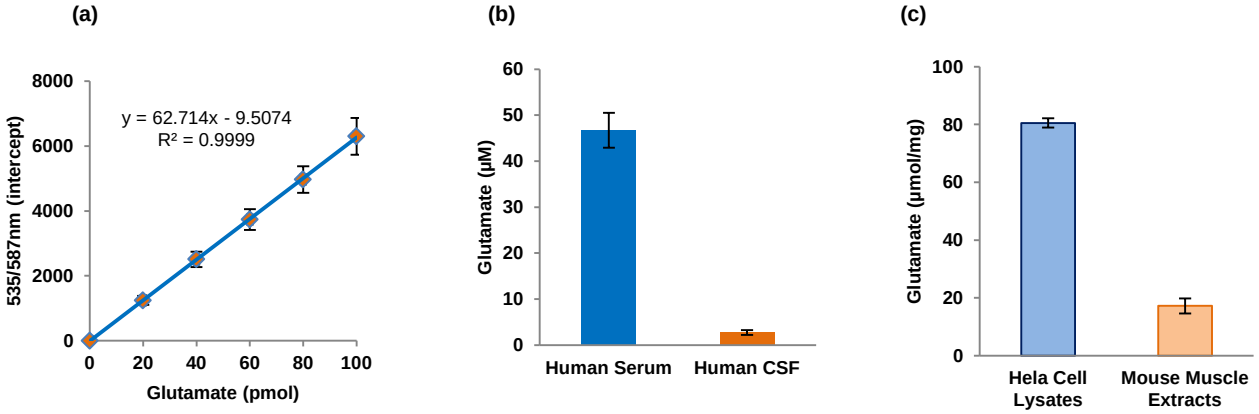


Figure: (a) Glutamate Standard Curve, results from multiple experiments. (b) Measurement of Glutamate amounts in Pooled Normal Human Serum (1:10; 5 µl) and Pooled Normal Human CSF (5 µl; undiluted). (c) Measurement of Glutamate amounts in Hela Cell Lysates (0.5 µg protein) and Mouse Muscle Extracts (0.5 µg protein). All assays were performed following kit protocol.

VIII. RELATED PRODUCTS:

Glutamate Colorimetric Assay Kit (K629)
Glutamine Colorimetric Assay Kit (K556)
DL-Serine Assay Kit (Fluorometric) (K743)
Phenylalanine Fluorometric Assay Kit (K572)
Glycine Assay Kit (Fluorometric) (K589)
Aspartate Colorimetric/Fluorometric Assay Kit (K552)
Glutamate Dehydrogenase Activity Colorimetric Assay Kit (K729)
Alanine Colorimetric/Fluorometric Assay Kit (K652)
Phenylalanine Assay Kit (Colorimetric) (K481)
Tyrosine Colorimetric Assay Kit (K573)
Cysteine Assay Kit (Fluorometric) (K558)
Dounce Tissue Homogenizer (1998)

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